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Detection of virus and bacterial con-taminants of semen of pedigree bulls with the use of molecular-genetic methods

The purpose. To tap contamination of deep-frozen se-men of pedigree bulls with virus and bacterial agents.

Methods. Bacteriological, molecular-genetic, statistical.

Results. 826 samples from 6 areas of Ukraine are examined. That has allowed tapping genetic material of virus.es of contagious rhinotracheitis and diarrhea of cattle in 3,5 and 6, 1 % of events accordingly. DNA of Chlamydia and mycoplasma was taped in 3, 15 and 8,6% of the examined samples accordingly. The belonging of virus of the diarrhea found in semen of bulls to 2 genotype of the causal organism is fixed

Conclusions. Considering existing risks of spread of communicable diseases through contaminated semen of bulls, there is a necessity of implementing mea-sures on profound quality control of genetic resources of cattle.

Key words: liquefrozen semen, bulls, virus and bacte-rial contamination, polymerase chain reaction, labora-tory diagnosis.

The solution of the problems of animal reproduction pathology nowadays is the key to the successful management of industrial livestock in Ukraine. Given the intensive introduction into the production practice of the newest biotechnologies for reproduction of animals and the wide international exchange of genetic resources, more attention must be paid to the effective control of sperm production, eggs and embryos at all stages of their receipt, freezing, transportation and use directly at farms. The technology of the modern breeding enterprise should be at the proper sanitary level and characterized by high fertilization efficiency irrespective of the terms of storage of frozen semen in liquid nitrogen [1]. Modern requirements to the sanitary quality of semen of bulls-pedigrees are aimed at preventing endometrial lesion of cows by pathogens of infectious diseases of viral and bacterial etiology during artificial insemination with deep-frozen sperm production. Adding components of animal origin (egg yolk, milk) to most commercial diluents used to freeze bulls' semen creates potential risks of contamination by bacteria, especially mycoplasmas, which threatens the spread of economically significant diseases of cattle [8, 17]. Consequently, attention should be paid to the control of genetic resources in breeding work in order to effectively break the epizootic chain. Most often, in the specimens of the sperm of the pedigree bulls, genetic material of

pesticides and herpesviruses, as well as chlamydia and mycoplasmas, are detected. Other pathogens include brucella, leptospira, campylobacteria, coccidia, leukemia viruses, parainfluenza-3 and Schmallenberg diseases. Previously, to exclude sperm contamination, the culture method was used, which is a rather costly and lengthy procedure with a low percentage of sensitivity [5]. The diarrhoea virus (VD) of cattle can maintain its activity in the semen of the pedigree boars for several months [10]. This virus can cause immunocompromised embryonic diseases, births of calves become persistently infected and are a constant source of transmission of the virus in the bovine population [7]. Chlamydial infections in ruminants have a wide range of symptoms, including polyarthritis, conjunctivitis, pneumonia and abortion [11]. By the spread of mycoplasmas and ureaplasm leads to international trade in animals, semen, embryos [16]. Among the rapid-use rapid tests, immunofluorescence and polymerase chain reaction (PCR) reactions were obtained. The latter is more sensitive and specific. It is believed that the study of biological material using molecular genetic methods is very expensive, but given the high sensitivity of the reaction patterns can be combined into a bullet, which significantly reduces the cost of the analysis. In addition, the timely detection of contaminated products can prevent significant economic losses in farms. The purpose of the research is to detect the contamination of deep-frozen semen of the pedigree bulls with infectious rhinotracheitis (IRT) viruses, leukemia, bovine leukosis, and chlamydia and mycoplasmas. Materials and methods of research. Frozen semen of cow seedlings in bordered granules or pails was delivered to a laboratory from various farms of Ukraine in Dewear's vessels at a temperature of - 196 ° C. Samples of sperm were examined for general microbial contamination, as well as the presence of genetic material of IRT, CD, leukemia, chlamydia and mycoplasmas using PCR [3] in accordance with protocols developed in the department of molecular diagnostics of the NSC "IECVM". Sequencing of the bovine genome of the bovine animal was carried out using the method of S. Vilcek [pers. comm.] at the University of Kosice (Slovakia).

Research results. During 2010-2014, 826 samples of frozen semen were examined. It was established that according to the biological indices, 11 series (11.8%) did not meet the requirements of GOST 287777 - 88 "Bullfish Sperm is frozen", while the sperm mobility after defrosting was 1.5-3 points in the acceptable norm not lower than 4 points (40% active sperm with rectilinear-translational motion), and the viability of gametes at the body temperature of the animal - not less than 5 hours. According to bacteriological studies of 93 series of sperm in the 21st series (22.6%) bacterial contamination was found (exceeding the number of microbial bodies per unit volume in one spermodozy in 3 - 4 times). For the conduct of molecular genetic studies, the presence of an IRL virus in bovine cattle has been confirmed in 3.5% of samples, and the RNA of cattle in RVD is 6.1% (table). The genetic material of chlamydia and mycoplasmas was found to be 3.15% and 8.6%, respectively, of the PCR results. Consequently, these results correlate with the data we obtained in 2008 [12] and significantly differ from the results of Russian scientists who detected the IRT virus in each 3rd sample of frozen semen of pedigree boars [2]. According to other literary data, the detection of IRT and VD viruses in the semen of pregnant women varies from 3 to 34% [6, 9, 15]. In Switzerland, the study of 304 samples of native and frozen semen of pedigree boars for PCR showed the presence of the genetic material of chlamydia in 20 cases (6.6%) [14]. The study of these samples of sperm production in relation to the presence of a pathogen of leukemia in cattle indicates the absence of specific products of PCR in the analyzed samples. This is an indication that there are no threats of infection of the cattle with the virus of leukemia during artificial insemination due to contaminated sperm production. At the same time, there are potential risks of the spread of the agent with sperm and embryos [12, 13].

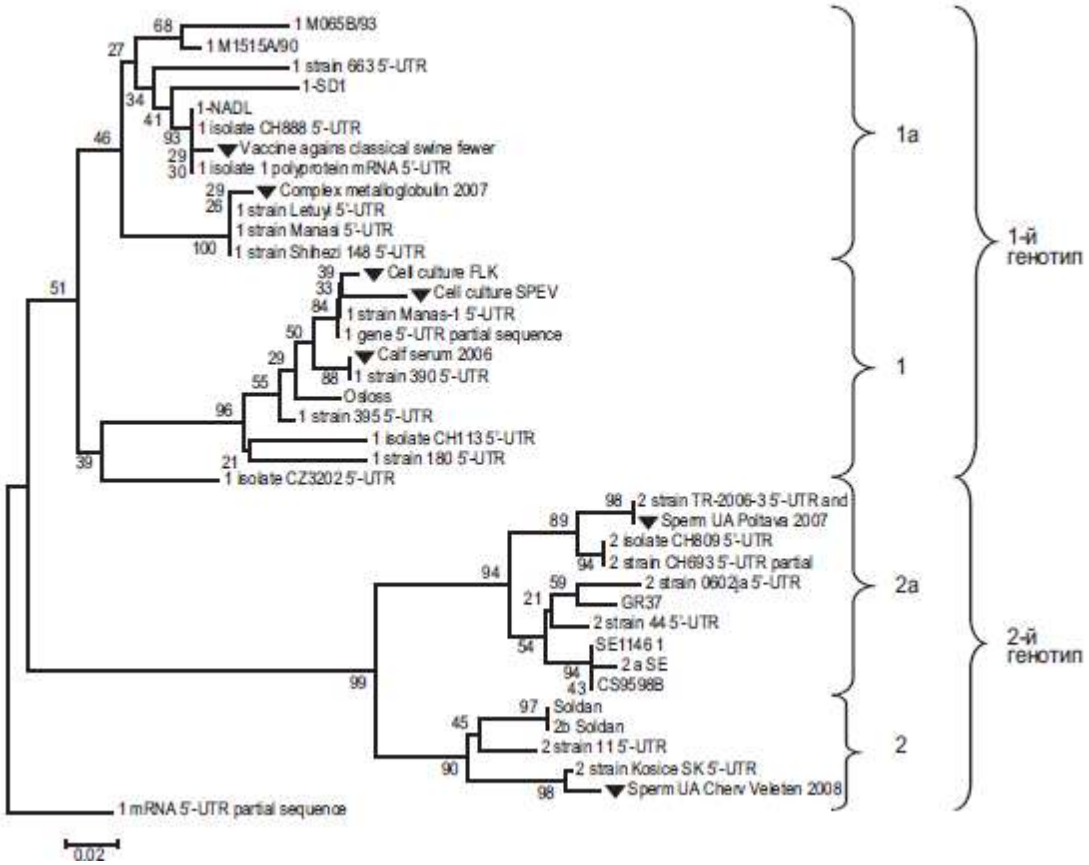
The resulting sequencing of nucleotide sequences of the 5'-UTR fragment length of 287 n. The samples of bovine cattle, found in semen, indicate the presence of two variants of the pathogen (intergroup distances of 0,200 - 0,373).

Results of the study of samples of frozen semen of bulls-breeders for 2010-2014 (n = 826)

Область	Досліджено разом	Виявлено позитивних зразків							
		вірус ІРТ		вірус діареї ВРХ		хламідії		мікоплазми	
		п.	%	п.	%	п.	%	п.	%
Запорізька	35	2	5,7	1	2,9	0	0,00	3	8,6
Київська	72	5	6,9	2	2,8	0	0,00	2	2,8
Полтавська	124	8	6,5	5	4,0	0	0,00	7	5,6
Дніпропетровська	93	3	3,2	7	7,5	3	3,23	8	8,6
Харківська	451	11	2,4	34	7,5	23	5,10	51	11,3
Сумська	51	0	0,0	1	2,0	0	0,00	0	0,0
Усього	826	29	3,5	50	6,1	26	3,15	71	8,6

Примітка. п. — позитивний зразок.

Built on the basis of the obtained dendrogram equation, the topographic features of the branching were split into 2 main lines containing strains of genotypes 1 and 2. Comminantes, detected in semen of the pedigrees, belonged to the genotype 2 (figure).



Phylogenetic relationships between strains of bovine diarrhea virus (Neighbor Joining, Bootstrap = 1000, - isolates of Ukrainian origin)

The isolates of Poltava and Cherv Veleten, found in samples of imported sperm production, belong to subtypes 2a and 2b, respectively. Their topographic position on the dendrogram indicated the affinity of the indicated contaminants with strains TR-2006 (Canada) and Kosice (Western Europe), respectively. The obtained data indicate that non-endemic variants of the virus already circulating in Ukraine are capable of causing mass diseases of calves and adult animals. Unfortunately, our state is not the only one among the European countries in which the recent

circulation of the genotype 2 virus has been registered (the virus of this genetic family was detected in Slovakia, France, the Netherlands and other countries). Detection of non-endemic variants of the bovine virus in Eurasia is a signal for strengthening measures to control its spread, including the development of diagnostic tools and monitoring systems, as well as the need for careful screening of veterinary surveillance facilities, especially genetic resources that may contain the identified type pathogen Consequently, molecular genetic research methods play an important role in the system of control over the genetic resources of cattle. In this case, PCR has a high analytical and diagnostic sensitivity in view of the fact that it is aimed at detecting not the pathogen itself, but only its genetic material. Employees of the Department of Molecular Diagnostics NSC "IEKVM" are constantly working on the development of new methods for the detection of sperm and semen contaminant bovine animals. Work in this direction has already made it possible to introduce a duplex version of the PCR for the simultaneous detection of the genetic material of Bovine Cattle and mycoplasmas [4]. Work is ongoing on a new system of real-time PCR-based monitoring of the genealogy of cattle.

Conclusions

According to the results of research of 826 samples of frozen semen of pedigrees, the genetic material of IRT and CD viruses was detected in 3.5 and 6.1% cases respectively. DNA chlamydia and mycoplasma were identified in 3.15 and 8.6% of samples, respectively. There is a lack of contamination of genetic resources causing leukemia in cattle, however, according to literature data, the risks of such contamination are high, which necessitates the introduction of measures for in-depth monitoring of the quality of sperm and embryos. The presence of pestiviral contaminants of semen of the pedigree to the second genotype is determined. Due to the existing risks of contamination of frozen semen of pedigree bulls with pathogenic viruses or bacteria during the development of a domestic standard for frozen semen it is necessary to provide for its virological and bacteriological control on IRT, VD, mycoplasmosis and chlamydiae using international laboratory methods of laboratory diagnosis (PCR, reactions immunofluorescence, isolation and identification of the pathogen).

Bibliography

1. Veterinary and sanitary requirements for semen in artificial insemination of animals / N.G. Balashov, AK Butakov, VN Rodina, NF Chuklov - M .: Kolos, 1977. - 220 p.

2. Infectious rhinotracheitis of cattle / AG. Glotov, AF Shulyak, GI Glotov, AN Sergeev // RASHN, Sib. Отд-ние, ГНУ ИЭВСиДВ. - Novosibirsk: IPI "Jupiter", 2006, - P. 196.

3. Polymerase chain reaction in the practice of veterinary medicine: science-method. manual / B.T. Stegany, AP Gerylovich, O.Yu. Liman and others; under ed. B.T. Stegnia and AP Gerylovich. - Kh .: NSC "IKEVM", 2006. - 110 p.

4. Development of duplex PCR for indicating mycoplasmas and bovine diarrhea viruses, followed by genotyping of the pathogen / AP. Gerylovich, IV Gorayichuk, VI Bolotin, O.S. Solodiankin // Visn. Ukr t-va geneticists and breeders. - Lviv, 2013. - Vol. 11, No. 1. - P. 28 - 33.

5. Farbotko G. E. Detection of viruses in semen of bulls / GE. Farbotko, NN Kondrachin // Veterinary Medicine. - 1992. - No. 5. - P. 47-48.

6. Analytical sensitivity of the assays used to detect bovine virus in the diarrhea virus in semen samples from the Southeastern United States / M.D. Givens, A.M. Heath, R.L. Carson et al .//Vet Microbiol. - 2003. - V. 96 (2). - P. 145 - 155.

7. Bolin S.R. Control of bovine virus diarrhoea virus / S.R. Bolin // Res. Sci Tech - 1990. - V. 9, No. 1. - P. 163 - 171.

8. Comparison of the bacteriological properties of various egg yolk sources and in vitro and in vivo fertilization potential of bovine semen frozen in egg yolk or lecithin based diluents / S. Bousseau, J.P. Brillard, B. Marguant-Le Guienne et al. // Thyriogenology. - 1998. - V. 50 (5). - P. 699 - 706.

9. Comparison of three polymerase chain reaction methods for routine detection of bovine herpesvirus 1 DNA in fresh bull sperm / C. B. Smits, C. Van Maanen, R.D. Glas et al .//Virol Methods. - 2000. - V. 85 (1 - 2). - P. 65 - 73.

10. Detection of bovine viral diarrhea virus in sperm obtained after inoculation of seronegative postpubertal bulls / M.D. Givens, A.M. Heath, K.V. Brock, B.W. Brodersen // Vet Res. - 2003. - V. 64 (4). - P. 428 - 434.

11. Isolation of an avian serovar of Chlamydia psittaci from a case of bovine abortion / H.U. Cox, P.G. Hoyt, R.P. Poston et al.//Vet Diagn Invest. - 1998. - V. 10. - P. 280-228.

12. PCR-screening of Chlamydiae and Viral Contamination of Bull Semen in Farms of Southern and Eastern Ukraine / A.P. Gerilovych, A.V. Skrypnyk, V.I. Bolotin et al .//Infektionen durch intrazellulare Erreger-Diagnostika, Epidemiologie und Pathgenese der Chlamydien- und Coxielleninfektionen: Zusammenarbeit mit dem NRL Q-Fieber und dem AVID (Jena, 25-26 September 2008). - Yena, 2008. - P. 39 - 41.

13. Potential risk of BLV distribution via bulls sperm / D. Beier, K. Bogner, A. Krisch et al. ///Proc. European virology association congress. - 2011. - P. 113.

14. Prevalence of chlamydiae in semen and genital tracts of bulls, rams and bucks / K. Teankum, A. Pospischil, F. Janett et al .//Theriogenology. - 2007. - P.

15. - P. 303-310. 15. Rapid detection of bovine herpesvirus in the semen of infected bulls by a nested polymerase chain reaction assay / S.A. Masri, W. Olson, P.T. Nguyen et al. // J. J. Vet Res. - 1996. - V. 60 (2). - P. 100 - 107.

16. Ureaplasma diversum as a cause of reproductive disease in cattle / B. Miller, A. Chelmonsky, B. Smits et al. //Food Animal Practice. - 1994. - V. 10. - P. 479-490.

17. Virulence and genotype of a bovine herpesvirus 1 isolate from a semen of a subclinically infected bull / J.T. van Oirschot, F.A. Rijsewijk, P.J. Straver et al .//Vet Res. - 1995. - V. 137 (10). - P. 235-239.

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